POCMiB016 BB/L013711/1
12 November 2015 – 11 November 2016
Funded by a Metals in Biology BBSRC NIBB Proof of Concept funding
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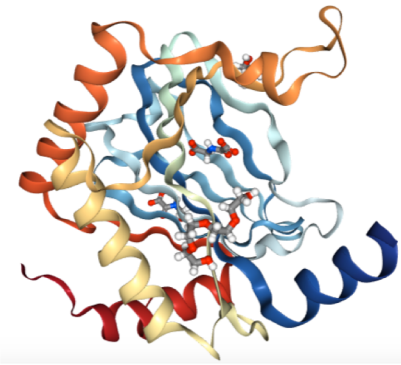
Proline hydroxylases for use in biocatalysis

"The project has revealed the substrate selectivity of oxygenases is much wider than we had expected, further highlighting their potential in biocatalysis for medicinal chemistry." Christopher Schofield, University of Oxford

Christopher Schofield & Michael McDonough, University of Oxford; Daniel Brookings, UCB Celltech

OUTCOMES: Our work concerned studies on a family of metal-dependent enzymes that add oxygen (or sometimes chlorine or bromine) atoms to drug-like small molecules and proteins. We focused on proline hydrolases, which in nature catalyse hydroxylation of the 5-membered ring amino acid proline. When we investigated the selectivity of the proline hydroxylases for different ring sizes and substitutions, we found that they can catalyses the hydroxylation of an unexpectedly wide range of rings, including bicyclic ring structures; some of these products are precursors for conversion into potential antibiotics. We also explored how these hydroxylases bind iron, using both assays for product formation and by X-ray crystallographic analyses of 'mutant' enzyme structures. Interestingly, we found that the proline hydroxylases can work with only two — rather than the normal three — points of attachment (ligand) of iron to the protein. These results inspired us to study metal binding by enzymes in cells (using mass spectrometry) and to study variations on iron binding by other types of hydroxylases. In one case we found the hydroxylase can work with only one protein ligand.



Cartoon of HIF prolyl hydroxylase 2 in complex with N-oxalylglycine (PDB-ID 5L9R)

INITIAL AIMS: Metal-dependent enzymes are incredibly powerful biological catalysts. In nature, biocatalysts can modify a common chemical scaffold to give several other products, which may have very different biological functions. Harnessing the power of such late-stage modification for drug discovery has potential to generate many molecules from a single drug candidate and could enable the efficient discovery of optimised molecules. There is a current lack of accessible libraries of oxygenase enzymes that are suitable for use in biocatalysts, and little information on how their activity is limited by metal binding in cells. We aim to develop proof of concept for the use of engineered proline hydroxylases for the stereoselective oxidation of substrates of choice.

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- Manuscript published: Zhang et al. (2017) PNAS 114: 4667- 4672
- Symposium on late-stage modification of pharmaceuticals
- Project expanded to include another potentially useful enzymes

Biotechnology and Biological Sciences Research Council